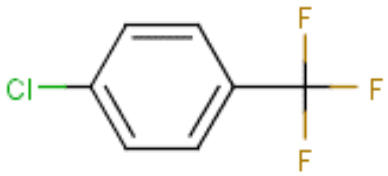
NTP Research Concept: 1-Chloro-4-(trifluoromethyl)benzene

Project Leader:
Matthew D. Stout, DIR/NTP/Toxicology Branch

Nomination Background and Rationale:



1-Chloro-4-(trifluoromethyl) benzene (*p*-chlorobenzotrifluoride; PCBTF) was nominated for toxicological testing by a representative from Kowa American Corporation, an importer, because of a lack of Occupational Safety & Health Administration (OSHA), National Institute for Occupational Safety and Health (NIOSH), or American Conference of Governmental Industrial Hygienists (ACGIH) exposure limits, despite its expanded use (particularly with stricter clean air act regulations) and therefore greater potential for exposure to not only workers but the general public. Additionally, in public comments submitted in 2001 on the National Toxicology Program (NTP) Center for Evaluation of Risks to Human Reproduction (CERHR) draft Expert Panel Reports on 1-bromopropane and 2-bromopropane, the Executive Director of the Institute for Research and Technical Assistance (IRTA) requested evaluation of existing toxicology data for PCBTF to determine if chronic testing was warranted. The basis for this concern was its increasingly widespread use as a volatile organic compound exempt solvent, specifically in automobile body coatings and parts cleaning. The NTP Interagency Committee for Chemical Evaluation and Coordination (ICCEC) evaluated the nomination in 2008 and recommended chronic toxicity and carcinogenicity and reproductive toxicity studies.

PCBTF has reported uses as a solvent in paint and coating formulations and in other applications, and as an intermediate in the synthesis of other chemicals, including herbicides. Although it has been reported that PCBTF is no longer produced in the US, it is imported in large quantities. The US annual production of PCBTF was reported at >10-50 million pounds in 1986, 1990, 1994 and 1998 and >1-10 million pounds in 2002 and 2006 (weight imported is included) by the EPA. PCBTF is an orphan high production volume (HPV) chemical. The VOC exempt solvent status and non ozone depleting properties have likely resulted in increased use of PCBTF as a solvent in end user applications, such as automotive refinishing and parts cleaning, where the potential for release of PCBTF and exposure via inhalation is relatively high. There are no standards in place to limit occupational exposure to PCBTF.

Structural Considerations: There are several chemicals with structures similar to PCBTF. Benzotrichloride (BTC) has been shown to be positive for *Salmonella typhimurium* mutations and is listed in the NTP Report on Carcinogens as reasonably anticipated to be a human carcinogen, whereas PCBTF and other benzotrifluorides (BTFs), including benzotrifluoride (BTF) and 3,4-dichlorobenzotrifluoride (3,4-DCBTF), have tested negative for salmonella mutations and have not yet been tested for carcinogenicity. BTF was nominated for NTP testing by the NCI in 2006, based on high production volume (1-10 million pounds in 1998), potential worker exposures, lack of

adequate toxicological data and demonstrated toxicity in short term studies, and by Kowa American in 2006 because of its use as a VOC exempt solvent. No US production was reported for BTF in 2002 or 2006 and NTP testing was deferred pending review of data collection under the Toxic Substances Control Act (TSCA) and the Organization for Economic Cooperation and Development (OECD) SIDS program. 3,4-DCBTF may be a good candidate for NTP testing, because of high production volume (1-10 million pounds in 2002), the presence of 2 chlorine atoms on the benzene ring, the higher predicted environmental persistence, and the limited available toxicity data; however, this compound, which has not yet been nominated for NTP testing, may be primarily used as herbicide intermediate with fewer dispersive uses and no US production was reported in 2006. Based on these considerations, PCBTF appears to be a reasonable candidate for testing of a representative BTF at this time.

Available Toxicity Information on PCBTF: The acute toxicity of PCBTF is low. Inhalation LC50s of ~3000- 4500 ppm in rats and ~2700 ppm in mice and oral LD50s of ~7-13 g/kg in rats and 11.5 g/kg in mice have been reported. There are prechronic toxicity data in the literature for both oral and inhalation exposure, including 13-week inhalation studies. In response to a previous nomination by the National Cancer Institute, the NTP conducted two-week oral gavage toxicity studies in rats and mice, toxicokinetic studies in male rats, and genotoxicity studies (http://ntp.niehs.nih.gov/go/TS-10472-T). Collectively, these studies suggest that the primary targets of PCBTF include the liver of both rats and mice and the kidney of rats. Centrilobular hypertrophy in the liver and alpha-2 microglobulin accumulation and hyaline droplet nephropathy in the male rat kidney have been consistently observed. There is some evidence of neurotoxicity from acute studies; however, a function observation battery and nervous system histopathology evaluation as part of the 13-week inhalation study in rats were negative. Carcinogenicity studies have not been reported. Limited data on reproductive toxicity is available in a summary of a non-guideline two-generation reproductive toxicity study from 1981. This study was limited by its design with only four weeks of exposure to the F0 generation and no functional assessment of the F1 generation and is not adequate to characterize the reproductive toxicity of PCBTF in rats. There are no data to evaluate developmental toxicity or immunotoxicity. The weight of evidence suggests that PCBTF is cytotoxic, but not genotoxic, to bacterial and mammalian cells; however, increased sister chromatid exchanges have been observed in mammalian cells.

Available Absorption, Distribution, Metabolism and Elimination and Toxicokinetic Information on PCBTF: After a single oral dose of [14C]-PCBTF in male (1 mg/kg) and female Sprague Dawley rats (1 and 104 mg/kg), 62-82% was expired unchanged, 3-4% was excreted (mostly unchanged) in feces, and 6-15% was excreted in urine. Urinary metabolites identified were glucuronides of dihydroxybenzotrifluoride and 4-chloro-3-hydroxybenzotrifluoride, with minor amounts of a mercapturic acid conjugate of PCBTF; unidentified metabolites constituted majority of the radiolabel excreted in urine. After 4 days ~ 1% of the dose remained in the body was found predominantly in the fat as PCBTF. Although sex and dose differences in the ADME of PCBTF were apparent, the small size of the study precluded definitive conclusions. Consistent with the results of

this study, hydroxylation of the benzene ring was predicted as a plausible metabolic pathway by the metabolism prediction program, METEOR (Lhasa Ltd, U.K.). Following gavage administration for two weeks to F344 rats and B6C3F1mice, sex and species differences in PCBTF concentrations in tissues were noted. Compared to female rats, male rats had higher concentrations in the liver, and much higher concentrations in the kidney, where relationship between PCBTF and alpha-2 microglobulin concentration was linear. PCBTF was not detected in mouse tissues perhaps due to lower levels in mice. Following inhalation exposure for 13 weeks, PCBTF accumulated primarily in fat. There was also evidence of modest P450 induction in this study; however, Vmax and Km values for the 3-hydroxylation of by liver microsomes from these rats were not significantly altered, providing evidence that PCBTF may not significantly induce P450.

Key Issues:
The use pattern, lack of occupational exposure standards and lack of chronic inhalation toxicity studies in experimental animals indicate that chronic inhalation toxicity and carcinogenicity studies in rats and mice are needed. In addition, an examination of the potential for PCBTF to induce developmental and reproductive toxicity is necessary to fill these data gaps.

Proposed Approach:

Specific Aims
Tier One:
Specific Aim 1. Conduct prechronic inhalation studies in rats and mice. These studies are necessary because there are no inhalation exposure data in mice, and the exposure concentrations in the reported study in rats do not appear to be sufficient to fully characterize the toxicity of PCBTF. These studies will be used to select exposure concentrations doses for the 2-year studies. Standard reproductive tissue histopathology and analysis of SMCVC data will be used to determine the potential for reproductive toxicity. An evaluation of the potential for PCBTF to induce alpha2μ-globulin nephropathy in male rats should be included.

Tier Two:
Specific Aim 2. Conduct 2-year toxicity and carcinogenicity studies in rats and mice.

Specific Aim 3. Conduct a teratology study in rats.

Specific Aim 4. A current guideline functional reproductive toxicology study in rats will be considered if data from the prechronic studies demonstrates the potential for reproductive toxicity. Since these studies are typically conducted via the oral route, ADME/TK studies may be needed to relate exposures to those occurring by inhalation.

Tier Three:
Specific Aim 5. An assay to evaluate toxicity to the developing reproductive, nervous and immune systems shall be considered. Inclusion of this study in the research

program would require further information on use patterns or reported toxicities as well as consideration of the data from tier one and two studies.

Significance and Expected Outcomes:
Although several BTFs have been reported as high production compounds and are similar in structure to BTC, which is reasonably anticipated to be a human carcinogen, there are no data to assess the chronic toxicity or carcinogenicity of the BTFs. Based on its production and use patters and reported toxicities, NTP testing of PCBTF is warranted to provide data on a representative of the BTF class of compounds. NTP studies on PCBTF will characterize the chronic toxicity and carcinogenicity of PCBTF following inhalation exposure for use in setting occupational exposure limits. In addition, this research program will provide an appropriate characterization of other toxicities, including developmental and reproductive toxicity.